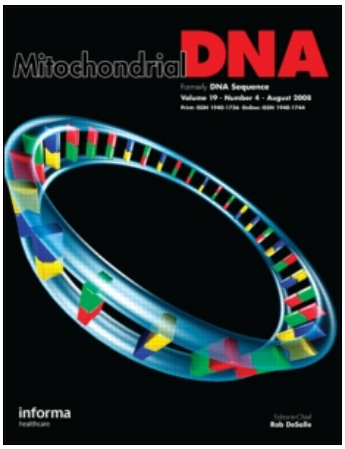


**Mitochondrial DNA**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640135

# Bluetongue virus serotype 17 sequence variation associated with neutralization

William C. Wilson [a]; Kristen A. Bernard [b]; Babara A. Israel [c]; James O. Mecham [a]
[a] Arthropod-Borne Animal Diseases Research Laboratory, USDA, ARS, Laramie, WY, USA [b] Arbovirus Laboratories, New York State Department of Health, Wadsworth Center, Albany, NY, USA [c] Department of Pathobiological Sciences, SVM, University of Wisconsin-Madison, Madison, WI, USA

First Published:June2008

**To cite this Article** Wilson, William C., Bernard, Kristen A., Israel, Babara A. and Mecham, James O.(2008)'Bluetongue virus serotype 17 sequence variation associated with neutralization',Mitochondrial DNA,19:3,237 — 240
**To link to this Article: DOI:** 10.1080/10425170701550524
**URL:** http://dx.doi.org/10.1080/10425170701550524

*DNA Sequence*, June 2008; 19(3): 237–240

informa
healthcare

**FULL LENGTH RESEARCH PAPER**

# Bluetongue virus serotype 17 sequence variation associated with neutralization

WILLIAM C. WILSON[1], KRISTEN A. BERNARD[2], BABARA A. ISRAEL[3], & JAMES O. MECHAM[1]

[1]*Arthropod-Borne Animal Diseases Research Laboratory, USDA, ARS, Laramie, WY 82071, USA,* [2]*Arbovirus Laboratories, New York State Department of Health, Wadsworth Center, Albany, NY 12159, USA, and* [3]*Department of Pathobiological Sciences, SVM, University of Wisconsin-Madison, Madison, WI 53711, USA*

*(Received 12 April 2007; accepted 3 July 2007)*



**Abstract**
Bluetongue virus (BTV) is an insect-transmitted orbivirus of importance to the cattle and sheep industry. The VP2 protein, encoded by L2, contains neutralizing epitopes. Previously, a panel of neutralizing monoclonal antibodies (MAbs) to the BTV serotype 17 (BTV-17) prototype strain was generated and it was determined that the neutralization domain consists of three overlapping epitopes. Over 30 amino acid changes were found between a neutralized BTV-17 prototype strain and a non-neutralized BTV-17 198 strain. In this study, the L2 genes from eight additional strains, representing both the neutralized and non-neutralized groups of BTV-17, were sequenced to determine the degree of conservation of the previously characterized differences. Comparison of the deduced amino acid sequences showed that 91% (30/33) of the previously noted changes were conserved within each group. The sequence of the M5 gene that encodes VP5 was also examined, since this surface protein has also been shown to affect neutralization. No consistent changes were noted between the neutralized and non-neutralized groups of BTV-17 by analysis of the VP5 protein. Finally, the L2 sequences of five MAb neutralization escape mutants were determined to identify specific amino acids involved in neutralization and perhaps virulence. All five mutants contained 1–3 amino acid changes that were in close proximity to a previously described variable region. These amino acid changes likely define critical sites in the overlapping neutralization domains previously described. This is the first description of two BT virus populations that have distinct neutralization characteristics co-circulating in a defined geographical region.

**Keywords:** *Bluetongue virus, neutralization, MAR mutants, L2, M5*

**Database accession number:** *AY6360673, AY636075, AY636078, AY636077, AY636071, AY636072, AY636074, AY636076, DQ080915, DQ080913, DQ080914, DQ080910, DQ080911, DQ080912*

Bluetongue virus (BTV) is an economically important insect transmitted orbivirus (Family: Reoviridae) that causes disease in domestic and wild ruminants. The virus genome consists of 10 segments of double-stranded RNA encoding for seven structural and four non-structural proteins. Three minor proteins VP1, VP4 and VP6 and the genome are contained in an icosahedral inner core consisting of two major proteins VP3 and VP7. This inner core is surrounded by an outer capsid consisting of another two major proteins, VP2 and VP5 (Roy et al. 1990). The serotype and neutralization determinants are associated with VP2 encoded by L2 (Huismans and Erasmus 1981; Grubman et al. 1983; Kahlon et al. 1983). Nucleic acid sequencing studies of monoclonal antibody resistant mutants (MAR) have identified variable regions associated with neutralization (Gould and Eaton 1990; DeMaula et al. 1993; DeMaula et al. 2000; Jewell and Mecham 1994). An additional neutralization epitope has been identified using competition for monoclonal antibody binding with sequence-specific synthetic peptides (Hwang and Li 1993). A panel of seven MAbs to BTV-17 define a virulence associated marker composed of three overlapping neutralizing epitopes (Bernard et al. 1996). Comparison of L2 sequences from a neutralized and a non-neutralized strain showed 34 amino acid changes in VP2 (Bernard et al. 1997). Understanding of neutralization requires

Correspondence: W. C. Wilson, Arthropod-Borne Animal Diseases Research Laboratory, USDA, ARS, Laramie, WY 82071, USA. Tel: 1 307 766 3622. Fax: 1 307 766 3500. E-mail: william.wilson@ars.usda.gov

ISSN 1042-5179 print/ISSN 1029-2365 online 
DOI: 10.1080/10425170701550524

Table I. BTV serotype 17 strains used in this study.

| BTV strain | Origin | Isolation date | Neutralizaton | L2 accession number | M5 accession number |
|---|---|---|---|---|---|
| ATCC | Wyoming | 1962 | Yes | | *DQ080915* |
| 284 | Puerto Rico | 1990 | Yes | *AY636073* | |
| 298 | Puerto Rico | 1991 | Yes | *AY636075* | |
| 299 | Puerto Rico | 1991 | Yes | *AY636078* | *DQ080913* |
| 300 | Puerto Rico | 1991 | Yes | *AY636077* | *DQ080914* |
| 198 | Tobago | 1990 | No | | *DQ080910* |
| 199 | Costa Rico | 1990 | No | *AY636071* | *DQ080911* |
| 240 | Guatemala | 1990 | No | *AY636072* | |
| 283 | Puerto Rico | 1990 | No | *AY636074* | *DQ080912* |
| 285 | Puerto Rico | 1990 | No | *AY636076* | |

Neutralization refers to the ability of the monoclonal antibody panel to neutralize the virus. Accession numbers are provided for sequences new in this report.



characterization of field strains because no reverse genetic system is currently available for orbiviruses. In this study, the conservation of the amino acid changes in VP2 between neutralized and non-neutralized strains was examined. The possible role of VP5 in neutralization was also examined by sequence analysis of the M5 genome segment. Finally, the nucleic acid sequences of L2 from five MAR mutants generated from a neutralized strain using five different MAbs were determined to define specific amino acids involved in neutralization.

The BT viruses exotic to the US used in this study were obtained from the Inter-American Bluetongue Project (Mo et al. 1994). The US BT viruses were obtained from the ABADRL reference collection. Summary histories for the BTV serotype 17 strains are shown in Table I. The dsRNA template was prepared using a differential lithium chloride precipitation procedure as described previously (Wilson et al. 1990). Viral strains were cloned by RT/PCR using terminal primers essentially as described previously (Wilson 1994). Sequence data was obtained directly from PCR products and from cloned PCR products using the commercially available TA cloning kit (Invitrogen, San Diego, CA). The sequence was obtained using standard automated sequencing (Applied Biosystems Inc., Foster City, CA). Sequence data were compiled with SeqMan software (DNA Star Inc., Madison, WI) aligned using Vector NTI (Informax Inc., Bethesda, MD) and phylogenetic analyses were performed using MacVector (Accerlys, Inc.).

The complete nucleic acid sequences of the L2 genome segment from eight additional BTV-17 strains (four strains for each of the previously identified MAb neutralized and non-neutralized groups (Bernard et al. 1996)) were determined. Phylogenetic analysis based on these sequence data clearly separated the two groups (data not shown). The predicted amino acid sequences from the neutralized virus strains were identical to that of the previously published ATCC prototype strain (Bernard et al. 1997) except for one consistent amino acid change at position 199. This region of the prototype strain was re-sequenced and this change was found to likely be a sequencing error in the previous report. The predicted amino acid alignment indicated that 30 of the 33 (91%) previously identified amino acid changes in the non-neutralized ATCC strain were the same within the non-neutralized virus group. Of the 30 amino acid changes that were consistent between the neutralized and non-neutralized strains, 10 were non-conservative and 10 were semi-conservative changes (Table II). Two of the non-conservative changes were within a previously defined hypervariable region (Bernard et al. 1997) based on previous epitope mapping studies (Gould and Eaton 1990; De Maula et al. 1993a; Hwang and Li 1993; Jewell and Mecham 1994; Pierce et al. 1995). Analysis of the predicted amino acid sequences revealed minor differences in hydropathic or hydrophilic profiles between the two L2 genotypes. Other secondary structure analyses were essentially identical.

Since, differences in neutralization can be affected by the other outer capsid protein, VP5 (Mertens et al. 1989; DeMaula et al. 2000), the sequence of the M5 gene that encodes this protein from six strains (three strains from the neutralized group and three strains from the nonneutralized group of viruses) was also determined. Two distinct M5 genotypes were identified with 19 consistent nucleic acid differences (98% homology). However, unlike the L2 gene, there was no relationship between the M5 genotype and the neutralization phenotype.

Complete L2 sequences and the deduced amino acid sequences were determined for five previously generated MAR mutants (Bernard et al. 1996). There were a total of seven amino acid changes between the MAR mutants and the parent ATCC strain (Figure 1). Interestingly, all of the MAR mutants had changes near a previously defined hyper-variable region (Bernard et al. 1997). The five MAbs, used to generate these MAR mutants, define a large binding site with three distinct overlapping neutralization patterns: (1) MAR 4B.3A and MAR 7C.3A; (2) MAR 11B.3A and MAR 21B.3A; (3) MAR 13B.3A (Bernard et al. 1996). The sequence alignments

Table II. The 30 amino acid changes that are consistent between the neutralized and nonneutralized strains are listed with the non-conservative changes highlighted in bold.

| Position | Neutralized | Non-neutralized | Neutralized type | Non-neutralized type | Change type |
|---|---|---|---|---|---|
| 54 | K (Lys) | R (Arg) | Basic, +'ve charge | Basic, +'ve charge | Conservative |
| 157 | Y (Tyr) | H (his) | Aromatic, polar | Basic, +'ve charge | Semi-conservative |
| **197** | **Y (Tyr)** | **N (Asn)** | **Aromatic, polar** | **Amide, polar** | **Non-conservative** |
| **201** | **G (Gly)** | **E (Glu)** | **Hydrophobic, polar** | **Acidic, −'ve charge** | **Non-conservative** |
| 208 | V (Val) | A (Ala) | Hydrophobic, nonpolar | Hydrophobic, nonpolar | Conservative |
| **229** | **S (Ser)** | **P (Pro)** | **Hydroxyl, polar** | **Proline, nonpolar** | **Non-conservative** |
| **241** | **S (Ser)** | **I (Ile)** | **Hydroxyl, polar** | **Hydrophobic, nonpolar** | **Non-conservative** |
| 248 | D (Asp) | S (Ser) | Acidic, −'ve charge | Hydroxyl, polar | Semi-conservative |
| 254 | I (Ile) | V (Val) | Hydrophobic, nonpolar | Hydrophobic, nonpolar | Conservative |
| 265 | T (Thr) | A (Ala) | Hydroxyl, polar | Hydrophobic, nonpolar | Semi-conservative |
| 281 | N (Asn) | K (Lys) | Amide, polar | Basic, +'ve charge | Semi-conservative |
| 290 | M (Met) | I (Ile) | Sulfur cont., nonpolar | Hydrophobic, nonpolar | Semi-conservative |
| **320** | **S (Ser)** | **P (Pro)** | **Hydroxyl, polar** | **Proline, nonpolar** | **Non-conservative** |
| 330 | G (Gly) | A (Ala) | Hydrophobic, polar | Hydrophobic, nonpolar | Conservative |
| 464 | E (Glu) | D (Asp) | Acidic, −'ve charge | Acidic, −'ve charge | Conservative |
| **503** | **S (Ser)** | **L (Leu)** | **Hydroxyl, polar** | **Hydrophobic, nonpolar** | **Non-conservative** |
| 576 | T (Thr) | A (Ala) | Hydroxyl, polar | Hydrophobic, nonpolar | Semi-conservative |
| **644** | **R (Arg)** | **T (Thr)** | **Basic, +'ve charge** | **Hydroxyl, polar** | **Non-conservative** |
| **657** | **R (Arg)** | **C (Cys)** | **Basic, +'ve charge** | **Sulfur cont., polar** | **Non-conservative** |
| **668** | **T (Thr)** | **M (Met)** | **Hydroxyl, polar** | **Sulfur cont., nonpolar** | **Non-conservative** |
| 705 | S (Ser) | D (Asp) | Hydroxyl, polar | Acidic, −'ve charge | Semi-conservative |
| 708 | R (Arg) | K (Lys) | Basic, +'ve charge | Basic, +'ve charge | Conservative |
| 749 | W (Trp) | S (Ser) | Aromatic, nonpolar | Hydroxyl, polar | Semi-conservative |
| **794** | **L (Leu)** | **S (Ser)** | **Hydrophobic, nonpolar** | **Hydroxyl, polar** | **Non-conservative** |
| 803 | I (Ile) | M (Met) | Hydrophobic, nonpolar | Sulfur cont., nonpolar | Semi-conservative |
| 824 | N (Asn) | S (Ser) | Amide, nonpolar | Hydroxyl, polar | Semi-conservative |
| 838 | R (Arg) | K (Lys) | Basic, +'ve charge | Basic, +'ve charge | Conservative |
| 839 | V (Val) | I (Ile) | Hydrophobic, nonpolar | Hydrophobic, nonpolar | Conservative |
| 852 | V (Val) | I (Ile) | Hydrophobic, nonpolar | Hydrophobic, nonpolar | Conservative |
| 852 | I (Ile) | V (Val) | Hydrophobic, nonpolar | Hydrophobic, nonpolar | Conservative |



of the pattern 1 MAR mutants are identical (Figure 1): whereas each of the two MAR mutants in pattern 2 have distinct amino acid changes. MAR 11B.3A (pattern 2) is the only mutant to have more than two amino acid changes; however, it is similar to MAR 11B.3A (pattern 2) in a charge change near the previously defined hyper-variable region. Finally, the single mutant in pattern 3, MAR 13B.3A, has a unique sequence compared to the other four mutants.

The panel of neutralizing MAbs clearly defines BTV-17 strains with unique neutralization phenotypes and distinct VP2 amino acid sequences. Overall, the VP2 amino acid sequence among the field isolates examined in this study is very highly conserved. This suggests that either the double-stranded RNA genome or structure of protein–protein interactions may limit the amount of variation allowed for this protein. The finding that this neutralization pattern is not

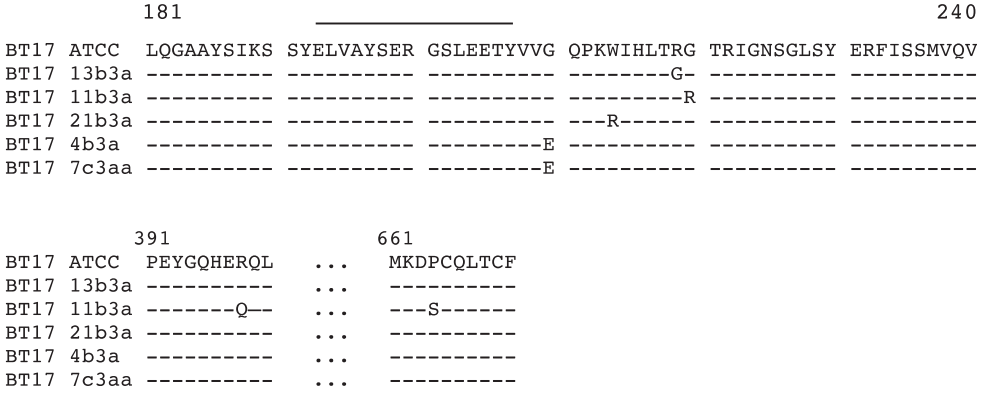


Figure 1. Comparison of predicted amino acid sequences from MAR mutants and the parent ATCC BTV-17 strain. The predicted amino acid alignment depicts only where the deduced amino acid sequences varied from the parent virus. The bar over the top of the sequence designates a previously defined cluster of amino acid variability.

affected by the VP5 genotype is contrary to previous reports (Mertens et al. 1989; DeMaula et al. 2000). This statement does not rule out the possibility that VP5 may influence other VP2 epitopes not evaluated in this study. Co-circulation of two conserved yet distinct BT VP2 neutralization and genetic types within a virus population has not been reported before. This suggests that there is an epidemiological advantage for the virus to maintain these distinct genotypes.

## Acknowledgements

The authors would like to thank Emily S. O'Hearn for technical assistance. The United States Department of Agriculture, Agricultural Research Service, Animal Health National Program, Project Number 5410-32000-011 and Veterinary Medical and Urban Entomology National Program, Project Number 5410-3200-014 provided funding for this project.

## References

Bernard KA, Israel BA, Schultz KT. 1996. A complex neutralization domain of bluetongue virus serotype 17 defines a virulence-associated marker. Viral Immunol 9(2):97–106.

Bernard KA, Israel BA, Wilson WC, Schultz KT. 1997. Sequence and cognitive analyses of two virulence-associated markers of bluetongue virus serotype 17. Intervirology 40(4):226–231.

DeMaula CD, Heidner HW, Rossitto PV, Pierce CM, MacLachlan NJ. 1993. Neutralization determinants of United States bluetongue virus serotype ten. Virology 195(1):292–296.

DeMaula CD, Bonneau KR, MacLachlan NJ. 2000. Changes in the outer capsid proteins of bluetongue virus serotype ten that abrogate neutralization by monoclonal antibodies. Virus Res 67(1):59–66.

Gould AR, Eaton BT. 1990. The amino acid sequence of the outer coat protein VP2 of neutralizing monoclonal antibody-resistant, virulent and attenuated bluetongue viruses. Virus Res 17(3): 161–172.

Grubman MJ, Appleton JA, Letchworth GJI. 1983. Identification of bluetongue virus type 17 genome segments coding for polypeptides associated with virus neutralization and intergroup reactivity. Virology 131:355–366.

Huismans H, Erasmus BJ. 1981. Identification of the serotype-specific and group-specific group antigens of bluetongue virus. J Vet Res 48:51–58.

Hwang GY, Li JK. 1993. Identification and localization of a serotypic neutralization determinant on the VP2 protein of bluetongue virus 13. Virology 195(2):859–862.

Jewell JE, Mecham JO. 1994. Identification of an amino acid on VP2 that affects neutralization of bluetongue virus serotype 10. Virus Res 33(2):139–144.

Kahlon J, Sugiyama K, Roy P. 1983. Molecular basis of bluetongue virus neutralization. J Virol 48:627–632.

Mertens PP, Pedley S, Cowley J, Burroughs JN, Corteyn AH, Jeggo MH, Jennings DM, Gorman BM. 1989. Analysis of the roles of bluetongue virus outer capsid proteins VP2 and VP5 in determination of virus serotype. Virology 170(2):561–565.

Mo CL, Thompson LH, Homan EJ, Oviedo MT, Greiner EC, Gonzalez J, Saenz MR. 1994. Bluetongue virus isolations from vectors and ruminants in Central America and the Caribbean. Am J Vet Res 55(2):211–215.

Pierce CM, Rossitto PV, MacLachlan NJ. 1995. Homotypic and heterotypic neutralization determinants of bluetongue virus serotype 17. Virology 209(1):263–267.

Roy P, Marshall JJA, French TJ. 1990. Structure of the bluetongue virus genome and its encoded proteins. Curr Top Microbiol Immunol 162:42–87.

Wilson WC. 1994. Development of a nested-PCR test based on sequence analysis of epizootic hemorrhagic disease viruses non-structural protein 1 (NS1). Virus Res 31(3):357–365.

Wilson WC, Fukusho A, Roy P. 1990. Diagnostic complementary DNA probes for genome segments 2 and 3 of epizootic hemorrhagic disease virus serotype 1. Am J Vet Res 51(6): 855–860.